*Creating viable muscle-motor neurone synaptic interactions in an *in vitro* 3D collagen co-culture gel model.

AST Smith[1], A Patel[1], B Kalmar[2], L Greensmith[2], V Mudera[3] and MP Lewis[1]

[1] *Department of Biomaterials and Tissue Engineering UCL Eastman Dental Institute.*
[2] *Department of Motor Neuroscience and Movement Disorders UCL Institute of Neurology.*
[3]*Division of Surgical and Interventional Sciences UCL Institute of Orthopeadics and Musculoskeletal Sciences.*

INTRODUCTION: The ability to generate biomimetic 3D skeletal muscle tissues *in vitro* would have important implications for both cell biology and medicine. To that end, a significant body of work has recently been published detailing the efforts of various groups to synthesise such a model. These protocols involve the seeding of muscle derived cells (MDCs) within a biomimetic scaffold of extra cellular matrix proteins and rely on the ability of MDCs to self-orientate between two fixed points. The contractile ability of our collagen based 3D model, constructed in such a way, is well established[1] and our goal now is towards making the model more biomimetic. Since denervated muscle quickly undergoes atrophy, it is likely that the introduction of a neural input will significantly improve the maturation of MDCs within our culture system, as well as establish a complex 3D co-culture model that better mimics *in vivo* conditions that exist within innervated muscle. Furthermore, the possibility of neuromuscular junctions (NMJs) formation *in vitro* opens the door to future research testing the effects of neuromuscular agents in culture, which may help to minimize the need for *in vivo* testing of such agents. Here we present our data characterising the development and maturation of this 3D co-culture system, compare results with both 2D and *in vivo* controls and discuss the implications for the future of skeletal muscle tissue culture techniques.

METHODS: MDCs isolated from P1 neonatal rat pups were seeded, at a density of 10^6 cells per ml, in neutralised type-1 rat tail collagen and plated, in 3 ml quantities, into standard dimension chamber slides (TTP Lab Tech). The slides each held a custom built (by engineers at the Eastman Dental Institute) floatation bar (termed "A-frame") at either end. Once the collagen gelled, it was cut away from the sides of the chamber and suspended in growth medium (20% fetal calf serum in low glucose DMEM). This provided only two focal attachment points for the gel so that, as the cells attached and contracted lines of isometric tension developed along the long axis of the gel. This tension provided sufficient mechanical stimulus to promote the alignment and fusion of the MDCs. The result was a 3D tissue possessing uniaxially aligned and fully differentiated myotubes capable of directed contraction. These models were cultured for 7 days before plating primary rat motor neurones, derived from E14 embryos, on top at a density of $4x10^5$ cells per gel. The gels were then cultured a further 7 days before being either stained for myogenic and neuromuscular junction markers or prepared for qPCR analysis.

RESULTS: Preliminary data demonstrated (i) the alignment of viable cells over the *in vitro* culture period (Fig. 1) and (ii) the ability of primary motor neurones to remain viable in this in vitro system when co-cultured with MDCs.

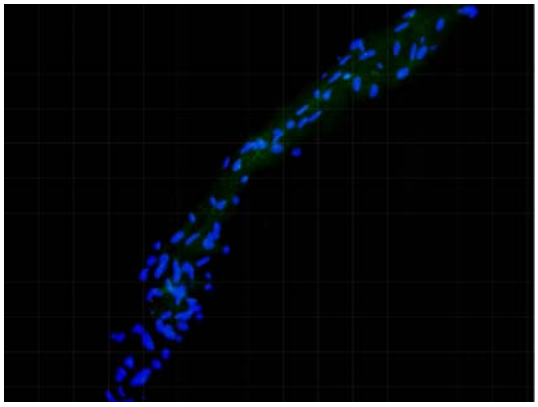

Figure 1: x20 image of cells within a 30µm slice of an in vitro *collagen matrix. Gels were stained for Desmin expression (green) and Nuclei (Blue).*

DISCUSSION & CONCLUSIONS: We have demonstrated for the first time the ability to co-culture primary motor neurones with mature myotubes in a 3D biomimetic culture system. We observed a significant improvement in muscle maturation in these 3D cultures compared to that previously reported for muscle cells *in vitro*. Although we have yet to establish the presence of functional NMJs, our results show that motor neurons are able to survive within the collagen 3D cultures.

REFERENCES: [1] M. Brady, M.P. Lewis, and V. Mudera (2008) *J Tissue Eng Regen Med* **2**:408-417.

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